

FIGURE 1: Annotation of GAS 40

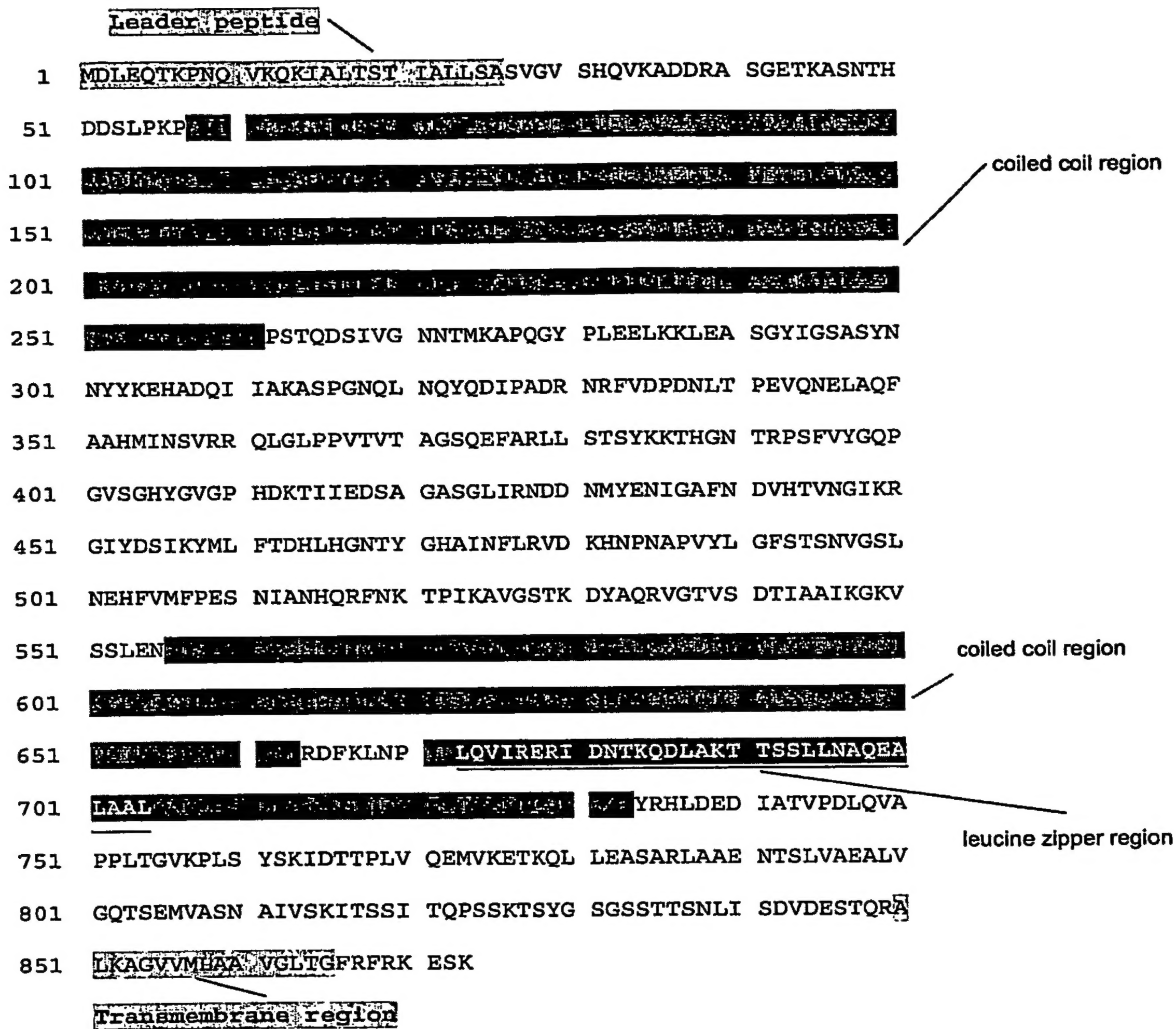


FIGURE 2 : Schematic of GAS40: putative surface exclusion protein prgA (873aa)

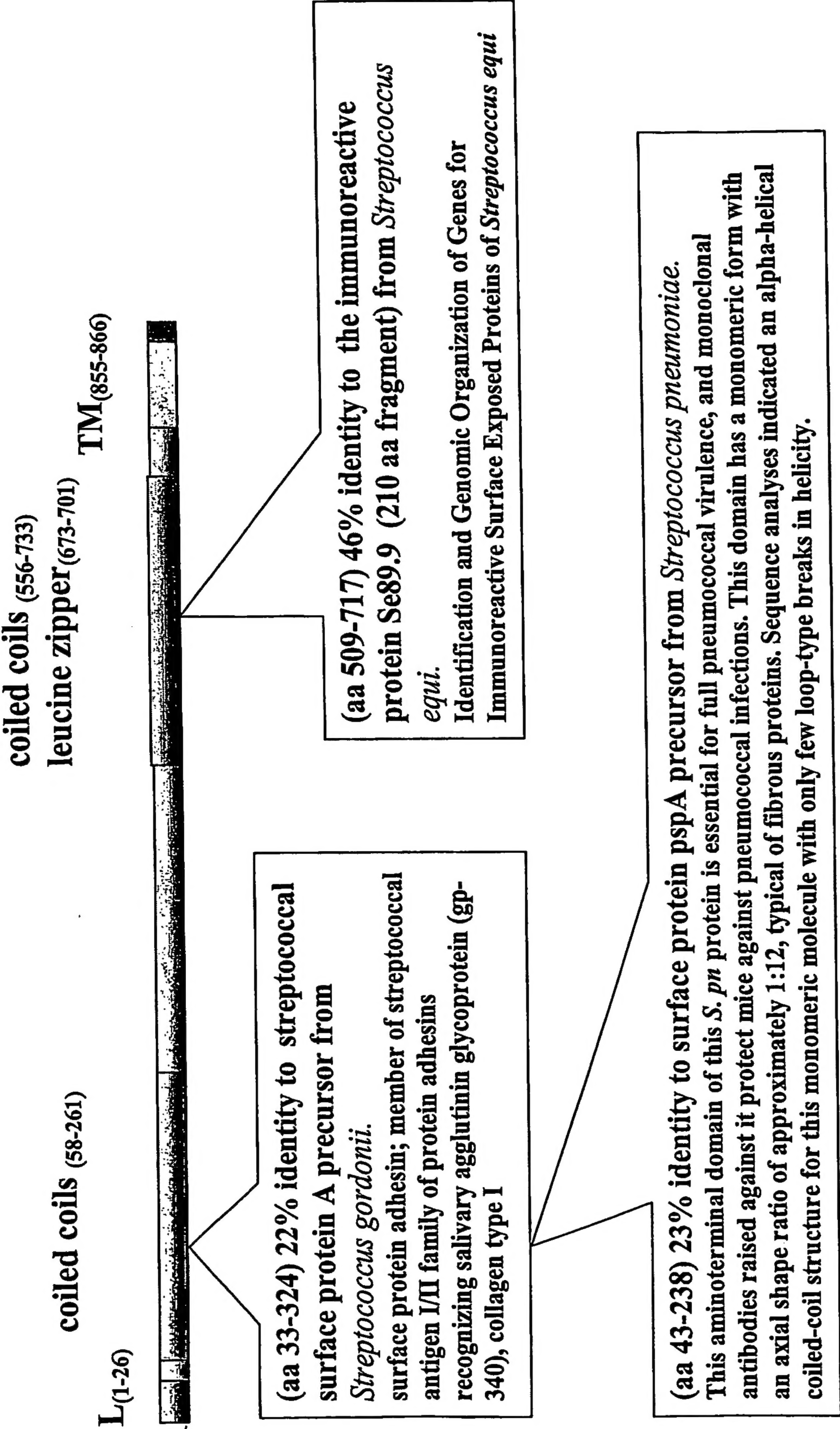


FIGURE 3: BLAST results of Coiled-Coil regions of GAS 40 with other *Streptococcus* bacteria

3(a) BLAST alignment of amino acid sequence of GAS 40 including the first coiled-coil region with SpA precursor of *Streptococcus gordonii*

```

>gi|25990270|gb|AAC44101.3| streptococcal surface protein A precursor
[Streptococcus gordonii]
      Length = 1575
>ref|NP_268623.1| putative surface exclusion protein [Streptococcus pyogenes]
      Length = 873

Score = 63.2 bits (152), Expect = 5e-11
Identities = 65/293 (22%), Positives = 124/293 (42%), Gaps = 13/293
(4%)

Query: 112 QDQTSKGTATTAAENAQKQAEIKSDYAKQA---EEIKKTTEAYKKEVEAHQAETDKIN
167
      Q + D+ + T A N + K + ++A + ++KT K E+ K
Sbjct: 33 QVKADDRASGETKASNTHDDSLPKPETIQEAKATIDAVEKTLSSQKAELETALATLTKTT
92

Query: 168 AENKAAEDKYQEDLKAHQAEVEKINTANATAKAEYEAKLAQYQKDLAAVQKANEDSQLDY
227
      AE +++ + KA + E A+++ A+ A++Q++L A + ++Q D
Sbjct: 93 AEINHLKEQQDNEQKALTSAQEIYTNLTASSEETLLAQGAHQRELTATETELHNAQADQ
152

Query: 228 QNKLSAYQAELARVQKANAEAKEAYE--KAVKENTAKNAALQAENEAIKQRNETAKANYD
285
      +K +A + A + A++ E K ++N AK A+ + +AI + +TA N
Sbjct: 153 HSKETALSEQKASISAETTRAQDLVEQVKTSEQNIAKLNAMISNPDAITKAAQTANDNTK
212

Query: 286 AAMKQYEADLAAIKKAKEDNDADYQAKLAAYQAELARVQKANADAKAAYEKAVEENTAKN
345
      A + E A ++ K +LAA +A LA + + K++ + N
Sbjct: 213 ALSSELEKAKADLENQKAKVKKQLTEELAAQKAALAEKEAELSRKSSAPSTQDSIVGNN
272

Query: 346 TAIQAEN---EAIKQRNAA---AKATYEAALKQYEADLAAAKKANEDSDADYQ 392
      T + E +K+ A+ A+Y K++ AD AK + + YQ
Sbjct: 273 TMKAPQGYPLEELKKLEASGYIGSASYNYYKEH-ADQIIAKASPGNQLNQYQ 324

```


FIGURE 3: BLAST results of Coiled-Coil regions of GAS 40 with other Streptococcus bacteria

3(b) BLAST alignment of amino acid sequence of GAS 40 including the first coiled-coil region with SpB precursor of *Streptococcus gordonii*

```
>gi|25055226|gb|AAC44102.3| streptococcal surface protein B precursor
[Streptococcus gordonii]
      Length = 1499
>ref|NP_268623.1| putative surface exclusion protein [Streptococcus
pyogenes]
      Length = 873
```

Score = 54.3 bits (129), Expect = 2e-08
 Identities = 53/226 (23%), Positives = 98/226 (43%), Gaps = 13/226 (5%)

```
Query: 111 QDQTSKGTATTAAENAQKQAEIKSDYAKQA----EEIKKTTEAYKKEVEAHQAETDKIN
166
```

```
      Q + D+ + T A N + K + ++A + ++KT K E+ K
Sbjct: 33 QVKADDRASGETKASNTHDDSLPKPETIQEAKATIDAVEKTLSSQKAELETALATKTT
92
```

```
Query: 167 AENKAAEDKYQEDLKAHQAEVEKINTANATAKA EYEAKLAQYQKDLAAVQKANEDSQLDY
226
```

```
      AE +++ + KA + E A+++ A+ A++Q++L A + ++Q D
Sbjct: 93 AEINHLKEQQDNEQKALTSAQEIYTNLTASSEETLLAQGAHQREL TATETELHNAQADQ
152
```

```
Query: 227 QNKLSAYQAELARV--QXXXXXXXXXXXXXXXXXNTAKNAALQAENEAIKQRNETAKANYD
284
```

```
      +K +A + A + + N AK A+ + +AI + +TA N
Sbjct: 153 HSKETALSEQKASISAETTRAQDLVEQVKTSEQNIAKLNAMISNPDAITKAAQTANDNTK
212
```

```
Query: 285 AAMKQYE---ADL---AAIKKAKEDNDADYQAKLAAYQAELARVQ 323
```

```
      A + E ADL A +KK + A +A LA +AEL+R++
Sbjct: 213 ALSSELEKAKADLENQKAKVKKQLTEELAAQKAALAEKEAELSRK 258
```

FIGURE 3: BLAST results of Coiled-Coil regions of GAS 40 with other *Streptococcus* bacteria

3(c) BLAST alignment of amino acid sequence of GAS 40 including the first coiled-coil region with Surface Protein PspA precursor of *Streptococcus pneumoniae*

```
>gi| 282335 |pir||A41971 surface protein pspA precursor - Streptococcus pneumoniae
>ref|NP_268623.1| putative surface exclusion protein [Streptococcus pyogenes]
```

Length = 873

Score = 48.1 bits (113), Expect = 6e-07
Identities = 46/200 (23%), Positives = 89/200 (44%), Gaps = 23/200 (11%)

```
Query: 139 KTKFNTVRAMVVPEPEQLAETK-----KKSEEAKQKAPELTKKLEEAKAKLEE-AEKK
190
```

```
          +TK +      +P+PE + E K      K + K + EL L + A++ E++
Sbjct: 43 ETKASNTHDDSLPKPETIQEAKATIDAVEKTLSSQQAELTELATALTKTTAEINHLKEQQ
102
```

```
Query: 191 ATEAKQKVDAEEVAPQAKIAELENQVHRLEQELKEIDSESESEDYAKEGFRAPLQSKLDAK
250
```

```
          E K      A+E+      + E + + + +E+ +E+E + + + ++ L +
Sbjct: 103 DNEQKAL TSAQEIIYTN TLASSEETLLAQGAEHQRELTATETELHNAQADQHSEK TALSEQ
162
```

```
Query: 251 KAKLS----KLEELSDKIDELDAEIAKLEDQL-----KAAEENNNVEDYFKEGLEKTI
299
```

```
          KA +S      + ++L +++ + IAKL +      KAA+ N+      LEK
Sbjct: 163 KASISAETTRAQDLVEQVKTSEQNIAKLNAMISNPDAITKAAQTANDNTKALSSELEKA-
221
```

```
Query: 300 AAKKAELEKTEADLKKAVNE 319
```

```
          KA+LE +A +KK + E
Sbjct: 222 ---KADLENQKAKVKKQLTE 238
```

FIGURE 3: BLAST results of Coiled-Coil regions of GAS 40 with other Streptococcus bacteria

3(d) BLAST alignment of amino acid sequence of GAS 40 including the second coiled-coil region with SpB precursor of *Streptococcus gordonii*

>gi|23380384|gb|AAN18299.1| immunoreactive protein Se89.9 (fragment)
[Streptococcus equi]

Length = 210

>ref|NP_268623.1| putative surface exclusion protein [Streptococcus pyogenes]

Length = 873

Score = 173 bits (438), Expect = 4e-45

Identities = 98/209 (46%), Positives = 144/209 (68%)

Query: 1 ESDIVDATRFSTTEIPKSGQVIDRSASIQALTNDIASIKGKIASLESRLADPSSEAEVTA
60

ES+I + RF+ T I G D + + +++ IA+IKGK++SLE+RL+ EA++ A

Sbjct: 509 ESNIANHQRFNKTPKAVGSTKDYAQRVGTVSDTIAAIKGKVSSLENRLSAIHQEADIMA
568

Query: 61 AQAKISQLQHQLAAQAKSHKLDQQVEQLANTKDSLRTQLLAKEEQAQLKANLDKALAL
120

AQAK+SQLQ +L + +S L+ QV QL +TK SLRT+LLAAK +QAQL+A D++LA

Sbjct: 569 AQAKVSQQLQGKLASTLKQSDSLNLQVRQLNDTKGSLRTELLAAKAKQAQLEATRDQSLAK
628

Query: 121 LASSKATLHKLEAAMEEAKARVAGLASQKAQLEDLLAFEKNPNRIELAQEKVAAAKKALA
180

LAS KA LH+ EA E+A ARV L ++KA L+ L F+ NPNR+++ +E++ K+ LA

Sbjct: 629 LASLKAALHQTEALAEQAAARVTALVAKKAHLQYLRDFKLNPNRLQVIRERIDNTKQDLA
688

Query: 181 DTEDKLLAAQASLSDLQAQRARLQLSIAT 209

T LL AQ +L+ LQA+++ L+ +IAT

Sbjct: 689 KTTSSLLNAQEALALQAKQSSLEATIAT 717

Figure 4: Secondary Structure Prediction of GAS 40**Figure 4(a) Secondary Structure prediction alignment with GAS 40 amino acid sequence**

10 20 30 40 50 60 70
 | | | | | |
 MDLEQTKPNQVKQKIALTSTIALLSASVGVSHQVKADDRASGETKASNTHDDSLPKPETIQEAKATIDAV
 CCCCCCchhhHHhhhhHHHHhhccceeeEEEEcCCcCCCCcCCCCCCCCCCCCcHHHHHHHHHHHH
 EKTL SQQAELTELATALTKTTAEINHLKEQQDNEQKAL TSAQE IYNTLASSEETLLA QGAEHQRELT
 HHHhhccchHHHHHHHHHHHHHHHHHH
 TETELHNAQADQHSKETALSEQKAS I SAETTRAQDLVEQVK TSEQNI AKLNAMISNPDAITKAAQTANDN
 HHHHHHHHHHccccchHHHHhhhhccchhhHHHHHHHHHHHHHHHHHHHHhhcCcHHHHHHHHHHhhc
 TKALSSELEKAKADLENQKAKVKKQLTEELAAQKAALAEKEAELSR LKSSAPSTQDSIVGNNTMKAPQGY
 cHHhhcCCCCCceEcCCCCCCCCC
 PLEELKKLEASGYIGSASYN NYKEHADQI IAKASPGNQLNQYQDI PADRNRFVDPDNLTP EVQNELAQF
 CHHHHHHHhhCccceccchHHHHHHHHHHHHHHhhCchhhhhhhccCccccCCCCCCCCCchHHHHHHHH
 AAHMINSVRRQLGLPPVTVTAGSQEFARLLSTSYKKTGNT RPSFVYGQPGVSGHYGVGPHDKTI IEDSA
 HHHHHHHHHHHhCCCCceecCCCHHHHHHHHHhhccccCCCCCceEEEcCCCceeeccceCccCceEEEEcC
 GASGLIRNDDNMYENIGAFNDVHTVNGI KRG IYDSIKYMLFTDHLHGNTYGHAINFLRV DKNPNAPVYL
 CCCceecCCcHHhhhhccccccccCccccchHHHHHHhhheccccCccchHHheeeecCCCCCceEE
 GFSTSNVGS LNEHFVMPESNI ANHQRFNKTP I KAVGSTKDYAQRVGT VSDTIAAI KGKVSSLENRLSAI
 EEEecCccCCCCceccccccccchHHhhCccccCccCHHHHHHHchhHHHHHHHHhCccchHHHHHHHH
 HQEADIMAAQAKVSQ LQGLASTLKQSDSLNLQVRQLNDTKGSLRTELLAAKAKQAQLEATRDQSLAKLA
 HHHHHHHHHHHHHHHHHHHHHHHHHhhccCchHHhhhhhhCcCHHHHHHHHHHHHHHHHHHHHHHHHHHHHH
 SLKAALHQTEALAEQAAARVTALVAKKAHLQYL RDFKLNPNRLQVIRERIDNTKQDLAKTTSSLNAQEA
 HHHhCCCCCHHHHHHHHHHHHHHHHHHHHHHHHHHHHH
 LAALQAKQSSLEATIATTEHQLTLLKTLANEKEYRHLDEDIATVPDLQVAPPLTG VKPLSYSKIDTTP LV
 HHHHHHHhhCcceecchHHHHHHHHHHHHHHhhhhHHHHhhccCCCCCCCCCCCCceecCCHHH
 QEMVKETKQLLEASARLAAENTSLVAEALVGQTSEMVASNAIVSKITSSITQPSSKTSYSGSGSSTTSNLI
 HHHHHHHHHHHHHHHHHHHHHHHHHHHHHhhcchHHHHhhchhhccceEEecCCCCCCCCCccccCce
 SDVDESTQRALKAGVVMLAAVGLTGFRFRKESK
 cCCchHHHHHHhhccceeeEeccccceeeccC

Sequence length : 873

PHD :

Alpha helix	(Hh) :	525 is	60.14%
3 ₁₀ helix	(Gg) :	0 is	0.00%
Pi helix	(Ii) :	0 is	0.00%
Beta bridge	(Bb) :	0 is	0.00%
Extended strand	(Ee) :	63 is	7.22%
Beta turn	(Tt) :	0 is	0.00%
Bend region	(Ss) :	0 is	0.00%
Random coil	(Cc) :	285 is	32.65%
Ambiguous states (?)	:	0 is	0.00%

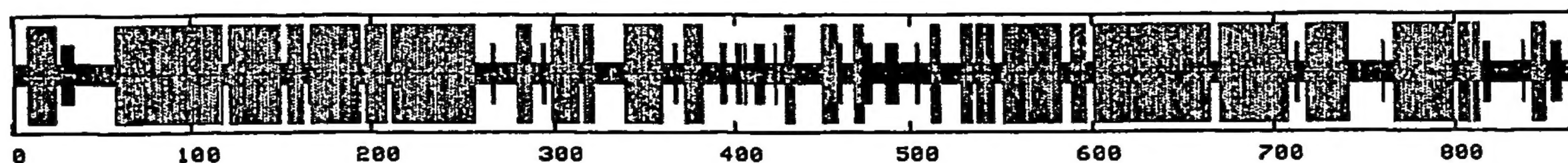
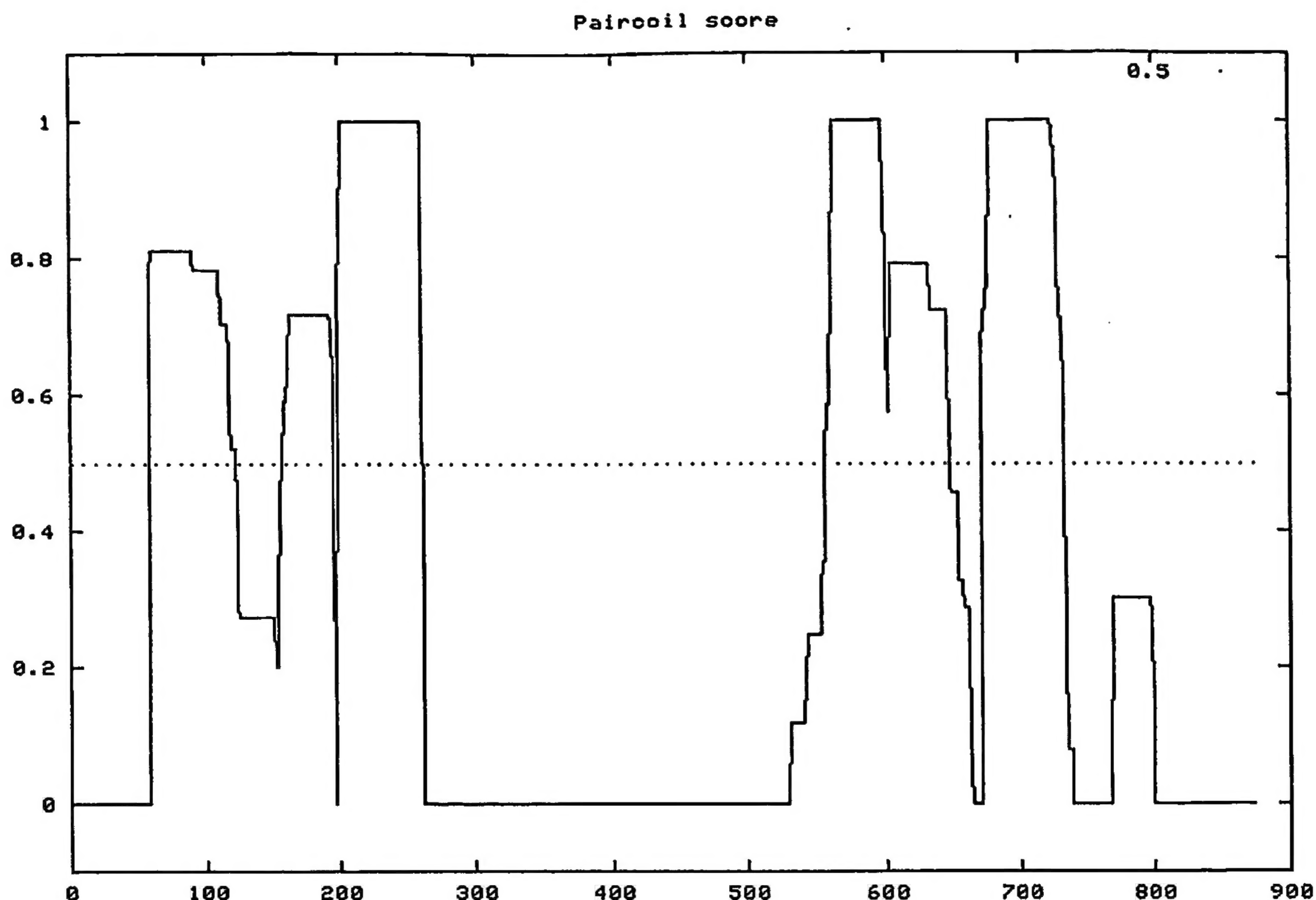


Figure 4(b): Secondary Structure prediction based on PairCoil Score



Coiled coils found:

positions	58- 121	initial register 'f'	probability	0.811
positions	156- 196	initial register 'e'	probability	0.720
positions	198- 246	initial register 'e'	probability	1.000
positions	247- 261	initial register 'a'	probability	1.000
positions	556- 646	initial register 'e'	probability	1.000
positions	671- 733	initial register 'f'	probability	1.000

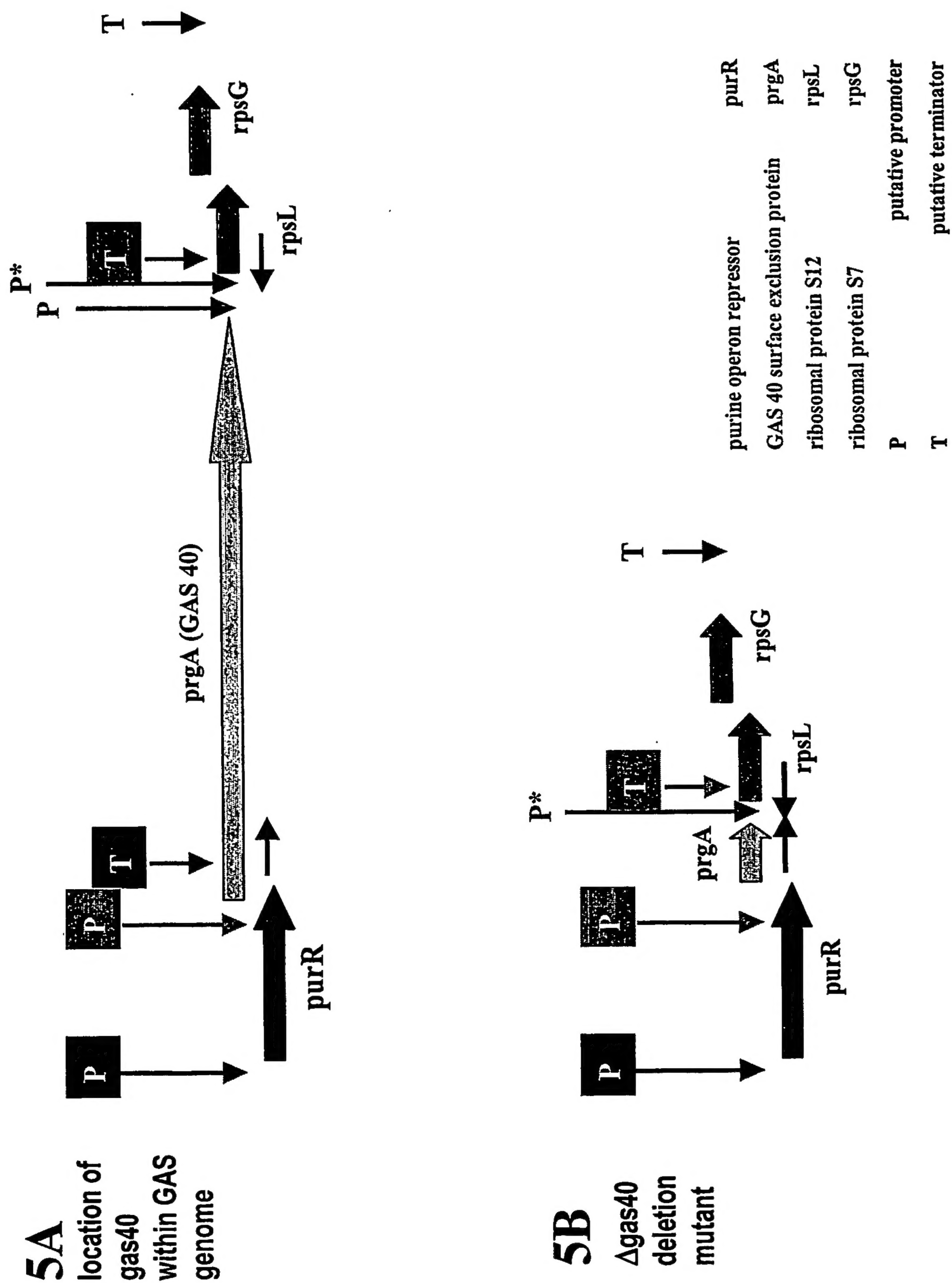
Figure 4(c): Secondary Structure prediction of Leucine Zipper within coiled coil.

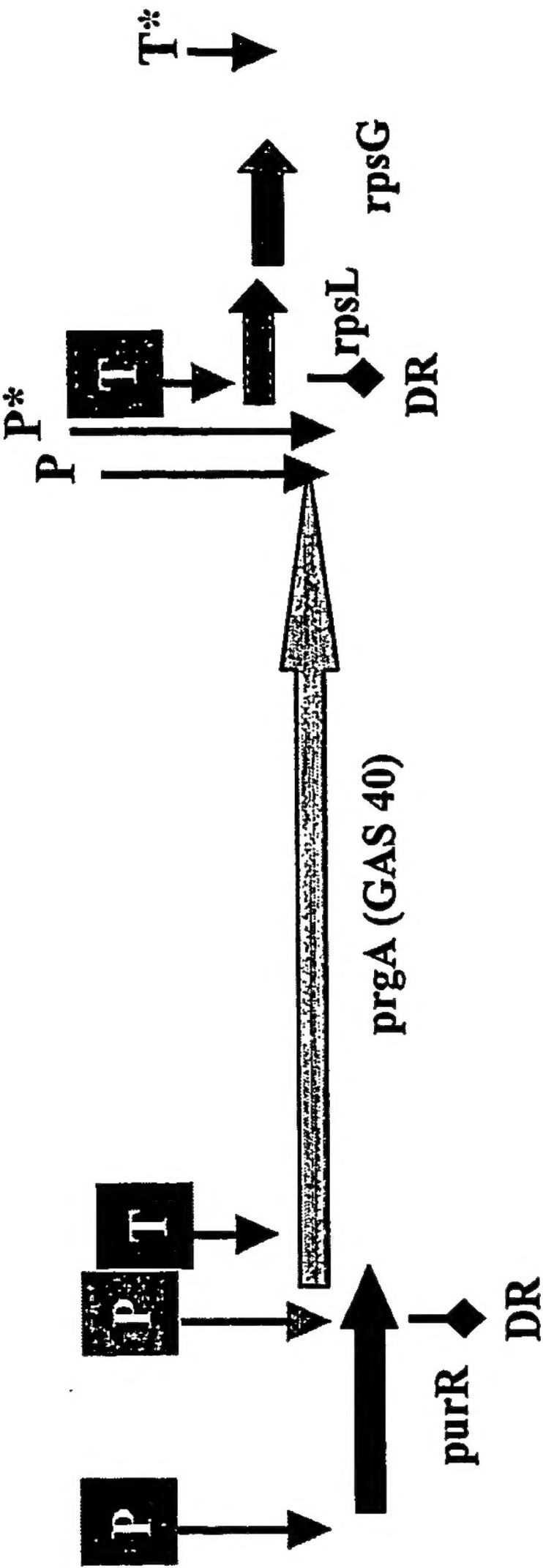
```

673                               701
QYLRDFKLNPNRLQVIRERIDNTKQDLAKTTSSLLNAQEALALQAKQSSLEATIATTEH
CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
L-----I-----L-----L-----L
OOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOO

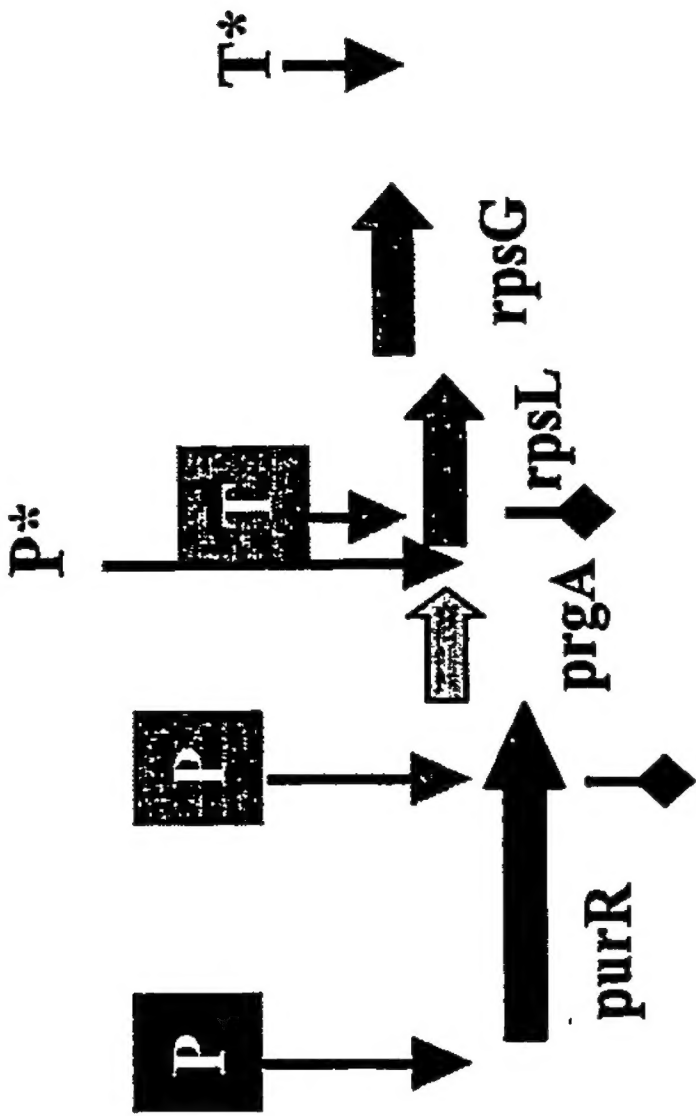
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Figure 5

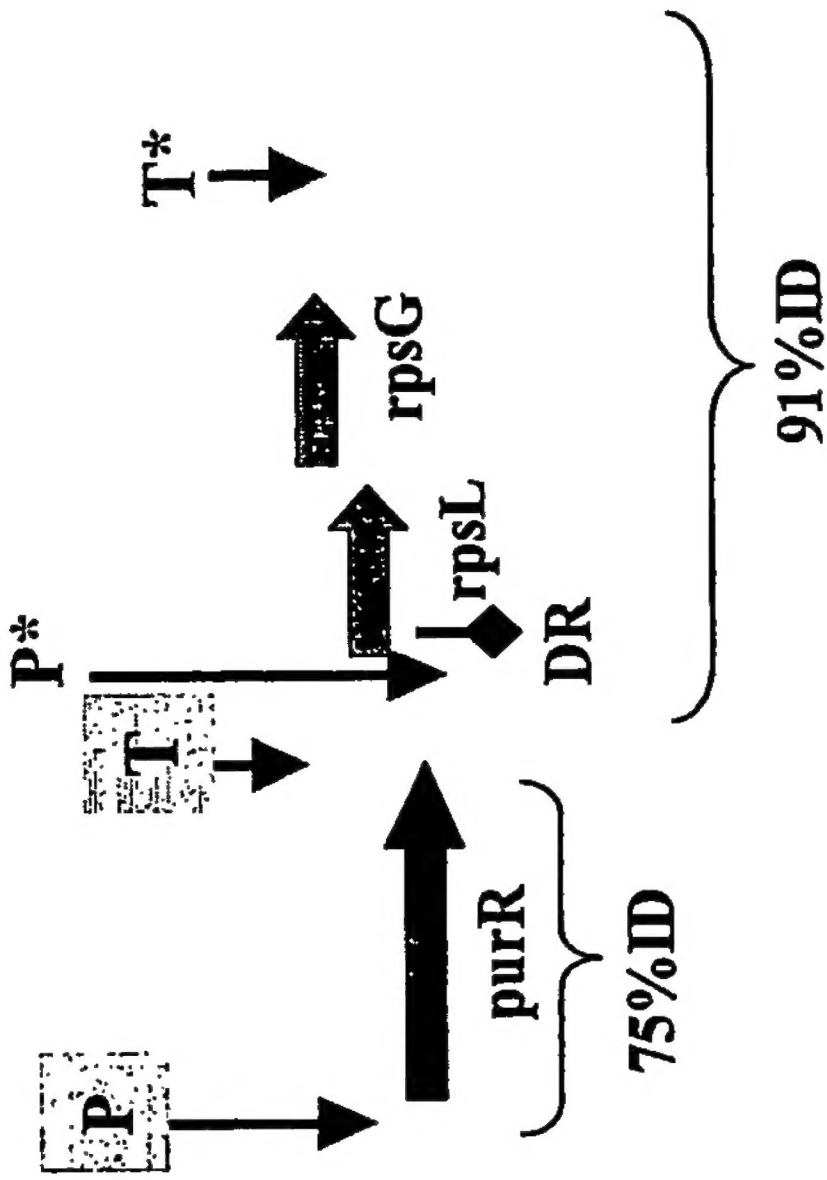




5C
direct repeats
surrounding GAS 40
within wild type genome



5D
location of direct
repeats within Δ gas40



5E
corresponding
genomic region
within GBS

FIGURE 6: FACS Comparison of GAS 40 in wild type GAS and GAS 40 deletion mutant

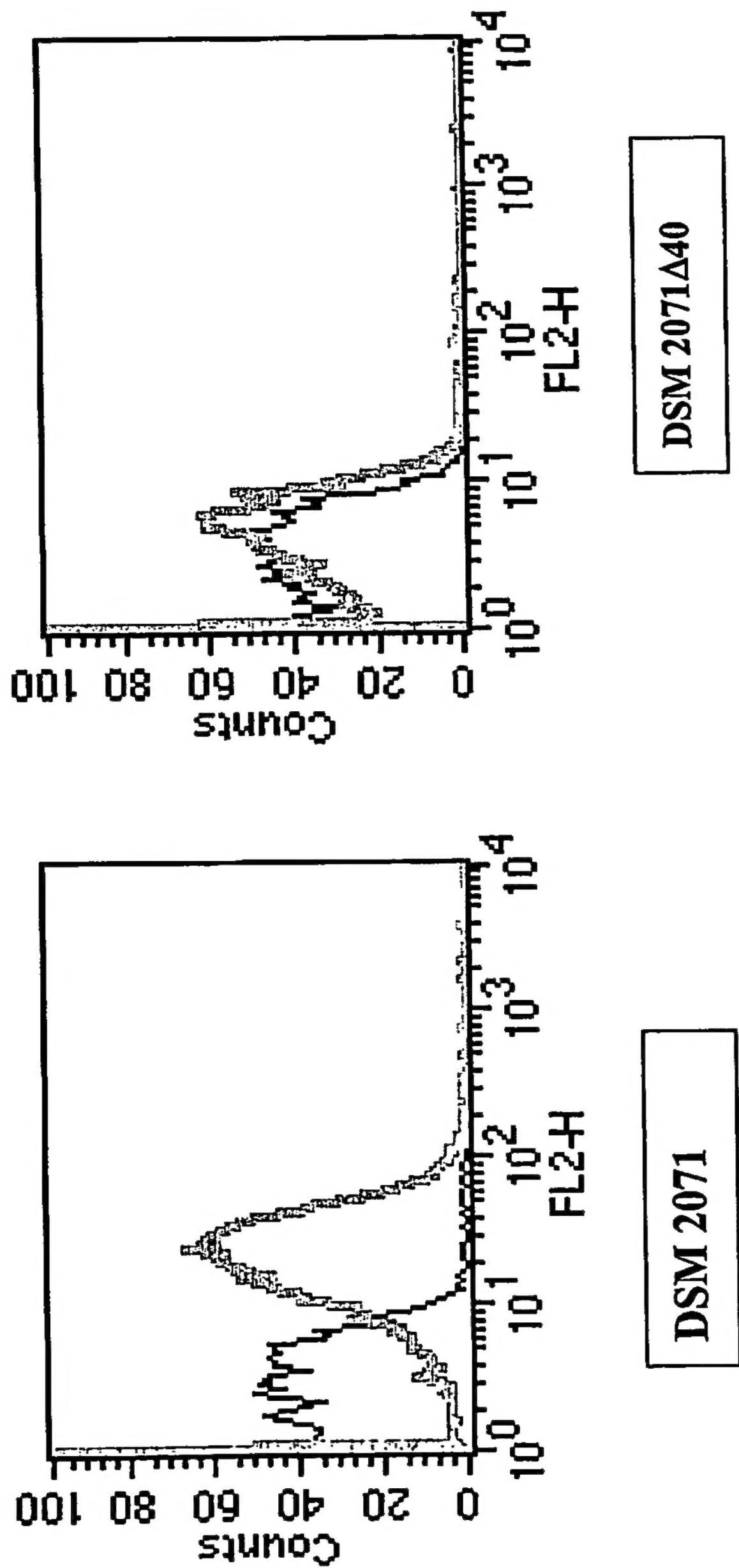
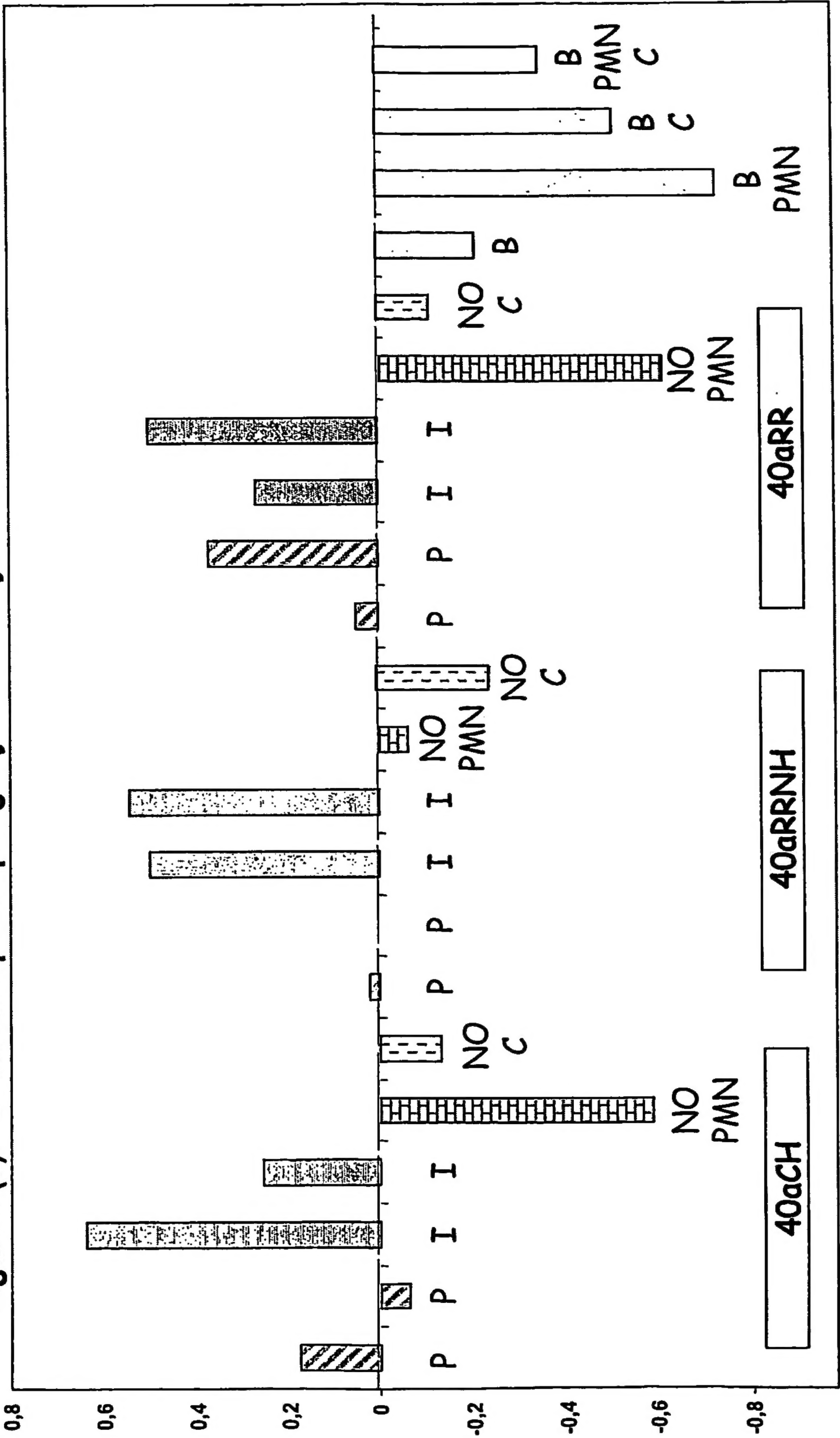


Figure 7(a): Bacterial opsonophagocytosis assay of GAS 40 constructs



B - bacteria
P - preimmune serum
I - immune serum
NO - without polymorphonucleates
C - without complement

Histogram bars represent the difference between logarithm at T 0 (initial time) and T60 (bacterial CFU counted after 60 minutes of incubation)

Figure 7(b): Bacterial opsonophagocytosis assay of GAS 40 constructs

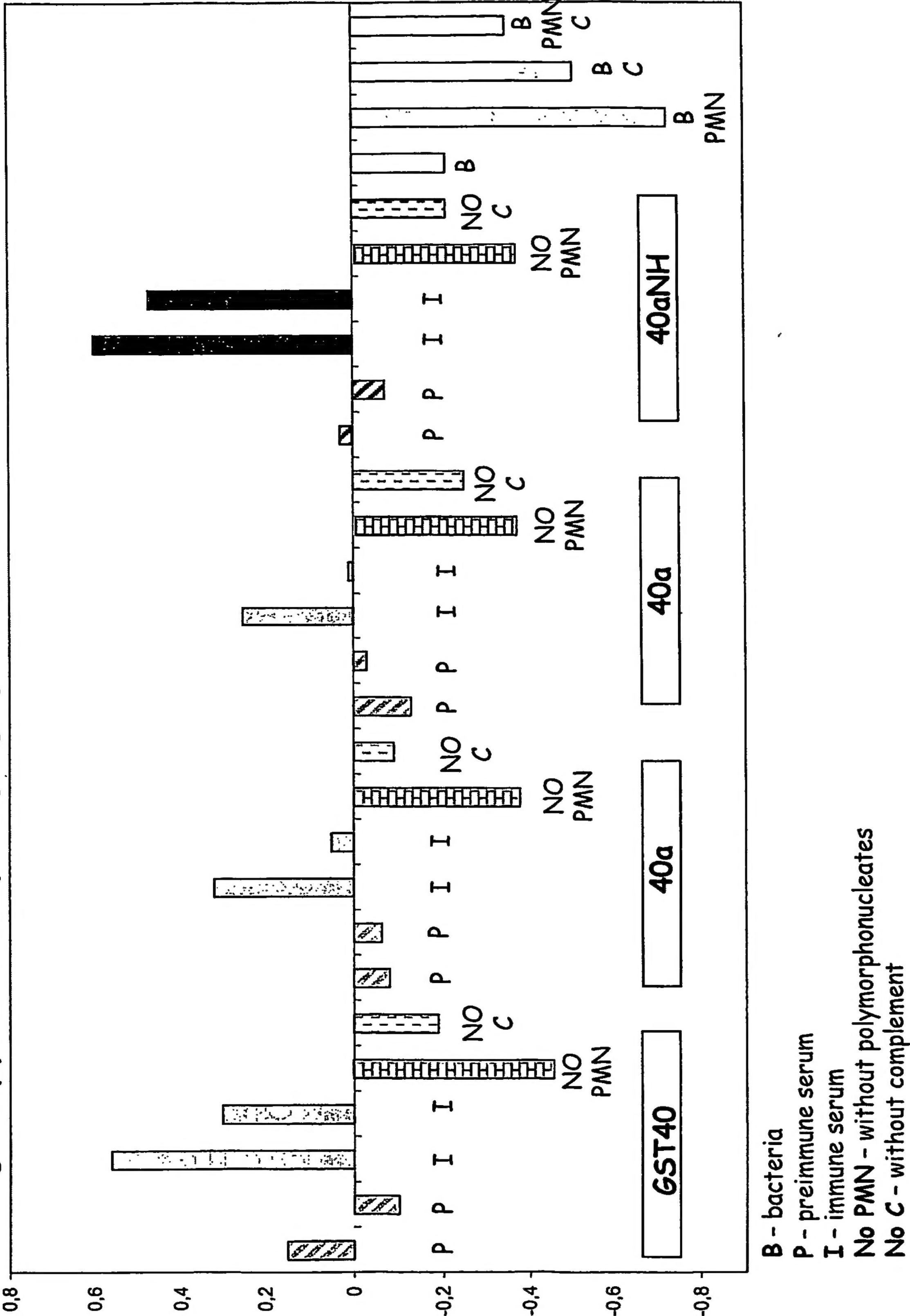


Figure 8: Immunization in Murine Mouse Model

GAS antigen	Survival/Tested mice			Protection	pValue	Protein Purity
	alive	dead	tested			
				%	Chi-square	%
gst 40	67	63	130	51	0.000012	
253	14	36	50	28	0.006	15
253-urea	2	8	10	20		25
253-gst	2	8	10	20		30
39	9	31	40	22.5	0.09	20
39a	13	37	50	26	0.016	10
39a	10	30	40	25	0.039	
39a	12	28	40	30	0.0046	
urea 366	21	78	99	21.2	0.046	65
117	19	51	70	27	0.0036	15
117-urea	1	9	10	10		80
117-urea-2M	7	23	30	23.3	0.1	80
117-urea-2M (prep 117)	8	32	40	20	0.2	
urea 504	9	31	40	22.5	0.09	50
504	14	26	40	35	0.0003	40
504	7	33	40	17.5	0.4	80
urea 389	7	23	30	23	0.1	30
533	14	56	70	20	0.12	50
new 533	4	16	20	20	0.34	30
gst 57	12	48	60	20	0.14	60
57a	0	20	20	0		50
294	17	73	90	18.8	0.14	80
130	15	65	80	18.7	0.17	40
130	7	23	30	23.3	0.1	40
84	8	32	40	20	0.2	70
urea 159	7	33	40	17.5	0.4	5
159a	2	8	10	20		65
527	10	40	50	20	0.17	50
527	3	17	20	15		80
217	7	33	40	17.5	0.4	50
511	13	67	80	16.2	0.41	80
277	8	42	50	16	0.52	5
277a	2	28	30	6.6		50
gst 202	3	17	20	10	0.75	5
202a	5	25	30	16.6	0.53	5
45	5	25	30	16.6	0.53	80
urea 309	5	25	30	20	0.53	8
290	6	34	40	15	0.67	50
529	6	34	40	15	0.67	5
gst 58	10	60	70	14.2	0.71	30
384	7	43	50	14	0.78	80
384RR	1	19	20	5		80
urea 509	7	53	60	11.6	0.84	50
509-NH	2	8	10			75
509-CH	0	10	10			75
193	7	53	60	11.6	0.84	65
urea 372	4	25	29	13.7	0.85	20
gst 42	4	26	30	13.3	0.9	50
95	5	35	40	12.5	1	55
urea 236	5	35	40	12.5	1	80
new 236	2	8	10	20		70
137	5	35	40	12.5	1	75
His-Stop	28	201	230	12.06		